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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A procaryotic cell system has been developed that can be used to determine the toxic action of chemicals acting at the level of the eucaryotic or procaryotic cytoplasmic membrane. Cell wall-less microbes known as mycoplasmas are used. In this current study, two perfluorinated fatty acids (C8 and C10) were found to inhibit the growth of the test mycoplasmas. Two apparent activities were observed. At high concentrations (10 mM) a detergent-like action was noted. At low concentrations (<10 mM) cell death was observed without detectable cell lysis. Altering the cell membrane (the presumed target of the toxic compounds) resulted in altered levels of toxicity. The nature of the toxic action of the perfluorinated fatty acids is currently being investigated using sodium dodecyl sulfate polyacrylamide gel electrophoresis, high performance liquid chromatography and microbiological procedures (such as selecting toxin resistant mutants).

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Membrane Alterations Following Toxic Chemical Insult-EQUIPMENT PROPOSAl (AFOSR-84-0246)

FINAL REPORT
TERMINATION DATE:
14 JAN 1986

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INTRODUCTION

The funds supplied by this grant along with "matching" funds from the State University of New York have been used to purchase major pieces of equipment for use on AFOSR and related scientific research. This report summarizes the results of this equipment grant. The specific equipment, vendor and price are listed. This summary is to fulfill a requirement of this granting agency. Also included is a brief summary of the research projects which are being supported by this reserach equipment.

EQUIPMENT PURCHASED

NAME	MANUFACTURER	COST
Speed-Vac Concentrator rotors, traps, and accessories	Savant Instruments, Hicksville, NY,11801	$ 3,000
Ultracentrifuge, L8-80M rotors: type 70Ti kit VTi80 kit Type 80Ti kit Type 19 kit Tube sealer kit Accessories	Beckman Instruments, Wakefield, MA, 01880	$50,937
High performance liquid chromatography system, includes: diode array detector 2 pumps pump controller fraction collector columns	LKB Instruments Paramus, NJ, 07652	$31,151
HPLC reservoir system	Kontes Glassware Vineland, NJ, 08360	$751
Spectrophotometer, uv/visable includes: model 4050 ultraspec controller model 4070 auto sampler temperature control unit dual channel recorder accessories	LKB Instruments, Paramus, NJ, 07525	$14,381
sample cells(micro and macro set)	Hellma Cells Jamaica, NY, 11424	$999

Densitometer [b] includes: laser densitometer chart recorder accessories	LKB Instruments, Paramus, NJ, 07625	$16,251
Computer support: [c] IBM PC Apple IIe Hardware and software for data analyses Printers	several vendors	$9,200
DEDUCTION FOR COST SHARING		$32,500
TOTAL FUNDS SPENT		$94,170

NOTES:

a) As per amendment dated 17, October 1984

b) The originally noted spectrophotometer (Beckman DU-8) had the potential to scan gels. Upon closer inspection, this feature was not worth the excessive price of the unit. The cost of the spectrophotometer and a stand-alone densitometer was lower than the planned total cost. The combination of equipment also gives me more versatility.

c) Two different computer systems were needed because no one system was compatible with the all of the equipment noted in this report.

PROJECT SUMMARY

This study involves using mycoplasmas, cell wall-less procaryotes , as the sentinel organisms to determine the toxic action of perfluorinated hydrocarbons. Three species of two different mycoplasma genera were used in this study. Acholeplasma laidlawii is a sterol non-requiring mycoplasma. Two members of the sterol requiring mycoplasmas (genus Mycoplasma) are also being used in this study. These species, M. capricolum and M. gallinarum, are more fastidious than A. laidlawii and they incorporate up to 10 times as much cholesterol into their membranes. This trio of strains represent similar yet different microbes with membranes of definable character which can be tested against toxins with possible membrane targets.

I have been studying the perfluorinated straight chain hydrocarbons, nonadecafluoro-n-decanoic acid (NDFDA) and pentadecafluoro-n-octanoic acid (PFOA). Initial work showed that, using a disc inhibition assay (similar to that used for antibiotic sensitivity testing) A. laidlawii was growth inhibited by these compounds and not by their counterpart fatty acid compounds , capric and caprylic acid, respectively.

Using sterile filter paper discs loaded with 25 ul of 100 mM of NDFDA, growth inhibition of the two Mycoplasmas species was less than 1 mm as compared to cleared zones of 2.0 mm or more when A. laidlawii was the test organism. At similar concentrations, no inhibitory zones were noted when capric acid was tested on A. laidlawii or M. capricolum. A small inhibitory zone (< 1 mm) was observed when the test organism was M. gallinarum.

Studies of the interaction of the toxin and the test cells in a liquid growth medium were also conducted. For Acholeplasma laidlawii propagated under low cholesterol growth conditions, the minimum inhibiting concentration of capric acid and NDFDA were determined to be 2.5 mM and 0.5 mM , respectively. When A. laidlawii was supplemented with horse serum (which increases the cholesterol content in the membranes) the minimum inhibitory concentrations were observed to be 5.0 mM for capric acid and 2.5 mM for NDFDA.

Similar tests using M. gallinarum and M. capricolum were performed. Both organisms were grown only in horse serum supplemented medium (defined as high cholesterol conditions). The established minimum toxic concentration of both capric acid and NDFDA was the same (2.5 mM) when M. capricolum was the test organism. When M. gallinarum was the test organism, it appeared that capric acid was more toxic than was NDFDA (5.0 mM as compared to 2.5 mM).

Preliminary studies in a broth culture growth system revealed that two actions of the perfluorinated fatty acids can be discerned. At concentrations of NDFDA greater than 10 mM, it appeared that the A. laidlawii cells were actually solublized (e.g. the turbidity of the cell-toxin mixture decreased). Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that polypeptides were lost from the toxin treated whole cells when compared to the untreated cells. The changes in SDS-PAGE profiles were similar to those seen when a detergent such as sodium dodecyl sulfate was used to treat the cells . In addition, alterations in polypeptide profiles due to treatment with the perfluorinated fatty acids were not similar to those seen when Proteinase K was used to treat the cells. Treating the cells with high concentrations of the non-perfluorinated fatty acid control chemicals produced similar "detergent-like" activity but at concentrations 5 to 10 times that of the perfluorinated compounds. Using 10 mM concentrations of NDFDA and PFOA, M. capricolum and M. gallinarum polypeptide profiles were not changed as compared to control samples.

ADDITIONAL STUDIES

Studies carried out in cooperation with Dr. Daniel Levitt of the Guthrie Research Institute, Sayre, PA are in progress. Dr. Levitt and I have found that 1 mM PFOA or NDFDA had a detergent-like activity on several tissue culture human cell lines. At lower concentrations (0.4 mM for NDFDA and 0.8 mM for PFOA) cells are growth inhibited but not solubilized. The exact toxic dose was dependent on the growth conditions used for the tissue culture cells. We shall be continuing these studies. These preliminary findings greatly parallel the results noted here for the mycoplasma studies.

Work with Dr. Alex Shrift of my Institution has begun in regard to defining the toxic action of the element selenium. Selenium is an essential micro-nutrient but, at higher concentrations, is toxic to many cells. Recently, a natural accumulation of selenium has turned a reservoir in California into a designated toxic waste area. Using a mycoplasma test system, I have found that mycoplasmas can tolerate levels of inorganic selenium typically toxic to most other bacteria. I am currently investigating the mechanism of this selenium tolerance. Preliminary data from this study has been used in an NIH grant proposal recently submitted by Dr. Shrift and myself.